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Chitobiase, a new reporter enzyme.

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N,N'-diacetylchitobiase (chitobiase) from the marine organism Vibrio harveyi is a highly stable reporter enzyme for gene fusions. This enzyme hydrolyzes the disaccharide chitobiose to N-acetyl glucosamine. The advantages of the reporter gene encoding chitobiase (chb) are: (i) that chitobiase and N-acetyl-beta-D-glucosaminidase activities are missing in E. coli strains, (ii) chitobiase can be monitored using blue/white colony indicator plates and (iii) convenient substrates for this enzyme are commercially available. The use of chitobiase as a reporter enzyme is generally applicable to the study of gene expression in those bacteria that do not contain N-acetyl-beta-D-glucosaminidases. We constructed plasmid vectors containing a multiple cloning site for producing in-frame fusions to chitobiase, the attP of lambda phase for movement into the bacterial chromosome for single-copy analysis, the gene encoding chloramphenicol acetyltransferase (cat), the pACYC184 origin of replication and the rrnBt1t2 terminator region upstream of the chb gene to prevent read-through from other promoters. In-frame fusions between the dnaA gene and chb were moved to the chromosome by site-specific recombination with the chromosomal attB site. These single-copy fusions were assayed for chitobiase to examine the effects of a deletion in the dnaA regulatory region.

PMID: 9863057 [PubMed - indexed for MEDLINE]